background often demonstrate strong genetic interactions (Qian et al., 2007; Yamamoto et al., 2008). These results suggest that while single core PCP genes act as the final common effectors of this pathway in vertebrates, other aspects of PCP are regulated through redundant genes, such as *Fzd3/6* and *Dvl1/2*. Moreover, a number of novel factors, including Cthrc1, have been recruited to act as important regulatory cofactors.

In summary, the results of the study by Yamamoto et al. (2008) identify the secreted collagen glycoprotein, Cthrc1, as a novel Wnt coreceptor that acts to specifically cluster Wnts with Ror2 and Fzd, leading to activation of the PCP pathway. These results further support the hypothesis that Wnts play a key role in vertebrate PCP and suggest that the conserved C-terminal region of Cthrc1 may provide valuable clues in the identification of additional Wnt cofactors.

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A New Kid on the TGFβ Block: TAZ Controls Smad Nucleocytoplasmic Shuttling

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A recent study from Varelas et al. in *Nature Cell Biology* reveals a role for the transcriptional regulator TAZ in TGFβ signaling. Not only does TAZ couple phospho-Smads to the transcriptional machinery, it is also essential for their nuclear accumulation.

Transforming growth factor beta (TGFβ) signaling controls diverse developmental processes and the pathogenesis of many diseases. A key step in TGFβ signaling is ligand-induced phosphorylation of R-Smads (Smad2/3 in TGFβ signaling; Smad1/5/8 in BMP signaling), which is mediated by serine/threonine kinase receptors. R-Smad phosphorylation allows their hetero-oligomeric complex formation with Smad4 and the nuclear accumulation of this complex, which ultimately regulates gene transcription in conjunction with a variety of transcriptional cofactors. Smad transcriptional cofactors have largely been thought to play a role in promoting signaling after Smads enter the nucleus (Figure 1; Feng and Derynck, 2005; Schmierer and Hill, 2007).

Although it has been reported that TGFβ favors nuclear import of phospho-R-Smads by enhancing their association with the nuclear import factor importin-β and/or disassociation from cytoplasmic retention factors such as SARA, live cell microscopy suggests TGFβ does not affect the nuclear import rate of Smad2 (Schmierer and Hill, 2007). In fact, accumulation of phospho-Smads in the nucleus in response to TGFβ has been shown to result from decreased Smad nuclear export, suggesting activated Smads may be held in the nucleus by retention factors (Schmierer and Hill, 2007). In the current issue of *Nature Cell Biology*, Jeffrey Wrana and colleagues (Varelas et al., 2008) present data suggesting that the transcriptional regulator TAZ/WWTR1 has an essential role in Smad nuclear retention, as well as in coupling Smads to transcriptional machinery.

An interaction between TAZ and Smad2/3 was initially found in an interaction screen (Barrios-Rodiles et al., 2005). Varelas et al. extended this finding and found that TAZ associated with heteromeric Smad complexes in a TGFβ-dependent manner. Knockdown of *TAZ* using siRNA markedly reduced TGFβ-induced transcription and upregulation of TGFβ target genes such as *Smad7* and *PAI-I*. TGFβ also stimulated binding of TAZ and Smad2/3 to the *Smad7* and *PAI-I* promoters, suggesting TAZ may be involved in TGFβ signaling at sites of Smad-mediated transcription.

So, how does TAZ influence TGFβ signaling? TAZ depletion did not interfere

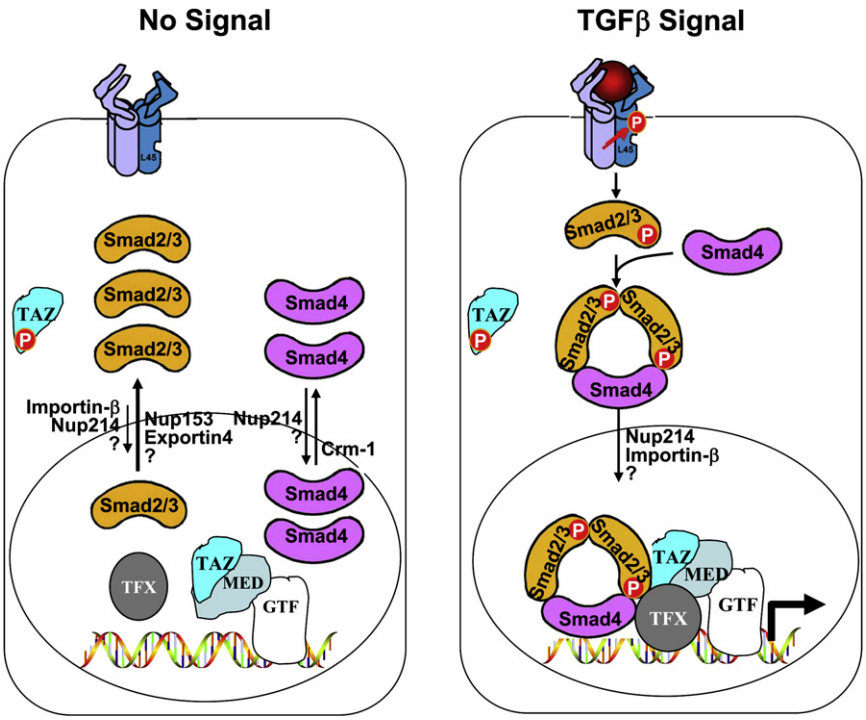

Figure 1. The Role of TAZ in TGFβ Signaling

In the absence of signal, Smads shuttle between the nucleus and cytoplasm without being recruited to the transcriptional machinery. During signal transduction, Smad2/3 are phosphorylated and form a complex with Smad4. TAZ binds to the heteromeric Smad complex to retain Smads in the nucleus, and couples them to transcriptional machinery via the ARC105 subunit of the Mediator complex (MED). TFX, context-dependent transcription factor X that cooperates with Smads; GTF, general transcription factor.

with Smad phosphorylation or heteromeric Smad complex formation. Instead, Smad2/4 nuclear accumulation was reduced in TAZ-depleted cells. Importantly, Smad nuclear localization and Smad-dependent transcription were restored in cells expressing siRNA-resistant TAZ. Because TAZ itself also engages in nucleocytoplasmic shuttling, this was exploited to confirm its role in regulating Smad localization. Expression of TAZ variants that could not be sequestered in the cytoplasm, or that could not shuttle into the nucleus, revealed that Smad localization correlates with that of TAZ.

So, what keeps TAZ in the nucleus to allow it to promote Smad accumulation and TGFβ-mediated transcription? The ARC105 subunit of the Mediator complex, which is important in TGFβ signaling (Kato et al., 2002), might be the answer. Varelas et al. found that TAZ interacted with ARC105 independently of TGFβ, and that these proteins showed extensive colocalization in the nucleus. Furthermore, ARC105 and Smad2 colocalized in the nucleus in response to TGFβ, and both bound the *Smad7* promoter in a TAZ-dependent manner. Together, data in this study suggest TAZ regulates Smad nuclear accumulation, and has a key role in coupling Smads to the transcriptional machinery via ARC105.

Like any major finding, this study poses several new and interesting questions. Because TAZ did not affect the Smad2 distribution in unstimulated cells, the data clearly suggest that TAZ's nuclear activity on TGFβ signaling is restricted to the active heteromeric Smad complex, consistent with the idea that the Smad complex may be held in the nucleus by retention factors in response to TGFβ. A role for Smad nuclear retention factors in TGFβ signaling is also supported by the fact that FoxH1—a transcription factor that interacts with Smads—can block Smad nuclear export (Xu and Massagué, 2004; Schmierer and Hill, 2007). However, the question remains as to how TAZ or other transcription factors, which presumably mask the nuclear export signal on Smads, release Smads during and/or upon termination of TGFβ signals. Given that TAZ-Smad interactions were dependent on TGFβ, it will be interesting to determine if the TAZ/ARC105-Smad complex disassembles as a result or prerequisite of Smad complex dissociation, and/or if it is triggered by R-Smad dephosphorylation by nuclear phosphatases such as PPM1A (Lin et al., 2006). Although TAZ couples active Smad complexes to the activation of TGFβ target genes, it will be relevant to establish whether TAZ plays a role in TGFβ-mediated active repression of genes. TAZ has been shown to contribute to the direct repression of PPARγ-dependent gene transcription, while simultaneously coactivating Runx2-dependent gene transcription to regulate mesenchymal stem cell differentiation (Hong et al., 2005). A clear mechanism underlying how chromatin-bound TAZ mediates transcriptional repression has yet to be revealed.

Although the BMP ligand induces TAZ expression (Hong et al., 2005), the results from Varelas et al. suggest an exclusive role for TAZ in TGFβ, but not BMP, signaling. Depletion of *TAZ* did not disrupt BMP-induced transcription, and only weak TAZ-Smad1 interactions were detected in response to BMP. Intriguingly, nuclear accumulation of the common Smad4 was altered by *TAZ* knockdown in response to TGFβ, but not BMP. These findings were supported by the effect of TAZ depletion in embryonic stem (ES) cells. In human ES cells, which depend on Smad2/3 signaling for pluripotency, differentiation to neuroectoderm occurred in the absence of TAZ. In contrast, knockdown of *TAZ* in mouse ES cells, which maintain pluripotency via the BMP pathway, did not alter pluripotent state. It will be worth investigating if other TAZ-like proteins (e.g., YAP) play a role in Smad nuclear accumulation in response to BMP family ligands. Notably, YAP can associate with Smad7 to enhance its ability to inhibit TGFβ signaling (Ferrigno et al., 2002).

Finally, there is also the added complication that TAZ itself binds to numerous proteins and is regulated by nucleocytoplasmic shuttling. Sequestration of TAZ in the cytoplasm by 14-3-3 proteins is dependent on its phosphorylation by protein kinase Lats in the Hippo signaling pathway (Lei et al., 2008), and now, it seems that TAZ is retained in the nucleus by ARC105. How is TAZ localization

controlled in the context of permitting Smad nuclear accumulation at an appropriate time? Intriguingly, TAZ overexpression also blocked phospho-Smad nuclear accumulation, suggesting appropriate TAZ levels may be critical in determining if Smad nuclear accumulation is permitted. Furthermore, the competition for TAZ between 14-3-3 and ARC105, influenced by the phosphorylation status of TAZ, may ultimately influence the nuclear duration of R-Smads. Regardless of the questions remaining, this study puts TAZ, phospho-Smads, and ARC105 together in the right place, at the right time, for successful TGFβ signaling.

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